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Calibration and functional analysis of three genetically encoded Cl-/pH sensors

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Abstract

Monitoring of the intracellular concentrations of Cl- and H+ requires sensitive probes that allow reliable quantitative measurements without perturbation of cell functioning. For these purposes the most promising are genetically encoded fluorescent biosensors, which have become powerful tools for non-invasive intracellular monitoring of ions, molecules and enzymatic activity. A ratiometric CFP/YFP-based construct with a relatively good sensitivity to Cl- has been developed (Markova et al., 2008; Waseem et al., 2010). Recently, a combined Cl-/pH sensor (ClopHensor) opened the way for simultaneous ratiometric measurement of these two ions (Arosio et al., 2010). ClopHensor was obtained by fusion of a red-fluorescent protein (DsRed-monomer) to the E2GFP variant that contains a specific Cl--binding site. This construct possesses pKa = 6.8 for H+ and Kd in the 40-50 mM range for Cl- at physiological pH (~7.3) As in the majority of cell types the intracellular Cl- concentration ([Cl-]i) is about 10 mM, the development of sensors with higher sensitivity is highly desirable. Here we report the intracellular calibration and functional characterization of ClopHensor and its two derivatives: the membrane targeting PalmPalm-ClopHensor and the H148G/V224L mutant with improved Cl- affinity, reduced pH dependence and pKa shifted to more alkaline values. For functional analysis, constructs were expressed in CHO cells and [Cl-]i was changed by using pipettes with different Cl- concentrations during whole-cell recordings. Kd values for Cl- measured at 33°C and pH ~ 7.3 were, respectively, 39 mM, 47 mM and 21 mM for ClopHensor, PalmPalm-ClopHensor and the H148G/V224L mutant. PalmPalm-ClopHensor resolved responses to activation of Cl--selective glycine receptor channels better than did ClopHensor. Our observations indicate that these different ClopHensor constructs are promising tools for non-invasive measurement of [Cl-]i in various living cells.

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Keywords

Fluorescent biosensors, Intracellular chloride, Intracellular pH, Non-invasive monitoring, Optogenetics